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Review Article

# Making the best use of our previous results as a clue for interpreting kinetics of scintigraphic agents

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Transport protein;
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**Summary** Up to now, we have performed scintigraphy with 201-thallium chloride (201-TlCl) and 99m-Tc-hexakis-2-methoxy-isobutyl-isonitrile (99m-Tc-MIBI) for malignant tumors and lymphoscintigraphy with 99m-Tc-rhenium-colloid (99m-Tc-Re) and 99m-Tc-human-serum-albumin-diethylene-triamine-penta-acetic-acid (99m-Tc-HSA-D) for lymph node metastasis. In this article, we re-evaluated scintigraphic images retrospectively with a hope that the results might be a clue, even if it is small, for dentists to try to improve the accuracy of diagnosis of malignant tumors. From scintigraphy, we obtained the tumor retention index as a factor to estimate the uptake of radioactive agents in tumor cells. Moreover, we estimated transport proteins of $Na^+/K^+$-ATPase and permeability-glycoprotein (P-gp) expressed on the cell membrane that might regulate the kinetic condition of radioactive agents. Among the tumor retention index, the transport protein and the histopathologic finding of tumors, there were relatively well correlations. The tumor retention index showed a difference clearly between malignant tumor and benign tumor. The transport protein revealed a distinct expression in accordance with the malignancy of tumor, and the uptake clearly depended upon the expression of transport protein. Moreover, the lymph node metastasis was detected well by lymphoscintigraphy with 99m-Tc-Re and 99m-Tc-HSA-D.



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**Contents**



## Introduction

Up to now, not a few radioactive agents have been introduced for the purpose of diagnosing malignant tumors of the head and neck, for example 67-Ga (gallium), 201-Tl (thallium), 99m-Tc (technetium), 198-Au (aurum), 131-I (iodine) and so forth. However, these radioactive agents are now not popularly used as before in routine examinations, because 18-F-fluoro-deoxy-glucose positron emission tomography (FDG-PET) is taking places of these radioactive agents. FDG-PET is a very superior method for malignant tumors [1]. At the time when FDG-PET has been introduced, we almost believed that most malignant tumors could be detected precisely and qualitatively with this method. However, this our expectation unfortunately ended with a fragile dream. This is not any all-purpose method. Even FDG-PET has some weak points. For example, FDG-PET is not able to distinguish malignant tumors from inflammatory lesions [2]. This radioactive agent shows almost the same accumulation in both malignant tumors and inflammatory lesions depending on its high sensitivity and affinity both to tumors and inflammatory tissues. This weak point is also an eternal, essential problem among usual tumor scintigraphies for a long time. Many researchers have tried to resolve this problem for a long time, but this is left unresolved. Against this problem, we also did in spite of a small ability. We focused our eyes on transport proteins of radioactive agents as one of means of solving this problem. We performed evaluations concerning several subjects, for example an expression of transport proteins on cell membrane and a relation of transport proteins with accumulation. Among the results of our evaluations, we searched to pick up some factors that seemed to be helpful and useful for diagnosing malignant tumors, and we tried to find out a possibility of qualitative diagnosis of malignant tumors of the head and neck using the factors [3—8]. In our scintigraphy for tumors, we usually employed 201-thallium chloride (201-TlCl) and 99m-Tc-hexakis-2-methoxy-isobutyl-isonitrile (99m-Tc-MIBI) as radioactive agents. We selected a couple of factors that control and closely relate with the uptake of these radioactive agents. We evaluated the expression of $Na^+/K^+$-ATPase and permeability-glycoprotein (P-gp) on the tumor cell membrane and the role of them as transport proteins in relation with both accumulation and washout of radioactive agents in tumor cells.

In this article, we re-evaluated retrospectively our results of tumor scintigraphy that we carried out until now. Moreover, we also re-evaluated lymphoscintigraphy with 99m-Tc-rhenium-colloid (99m-Tc-Re) and 99m-Tc-human-serum-albumin-diethylene-triamine-pentaacetic-acid (99m-Tc-HSA-D) [9—11]. With this thing and that, most data used in this article were quoted from some of our previous reports on journals [3—11] and modified to some extent.

## Scintigraphy

### Scintigraphy for tumors

#### Clinical evaluation of scintigraphy with 201-Tl

201-Tl was first used to evaluate the viability of the myocardium. After a while, this agent was introduced for the examination of malignant tumors of the head and neck [12,13]. In this section, we evaluated the usefulness of 201-Tl for malignant tumors of the head and neck.

*(Methods and materials of scintigraphy with 201-Tl).* We used 85 patients with a malignant tumor of the head and neck (squamous cell carcinoma) and 10 patients with a benign tumor (7 with pleomorphic adenoma and 3 with Warthin's tumor).

Intravenous injection of 74MBq of 201-Tl was performed. An early dynamic scan (for 5 min immediately after injection), a delayed dynamic scan and a spot scan (at 2.5 h after

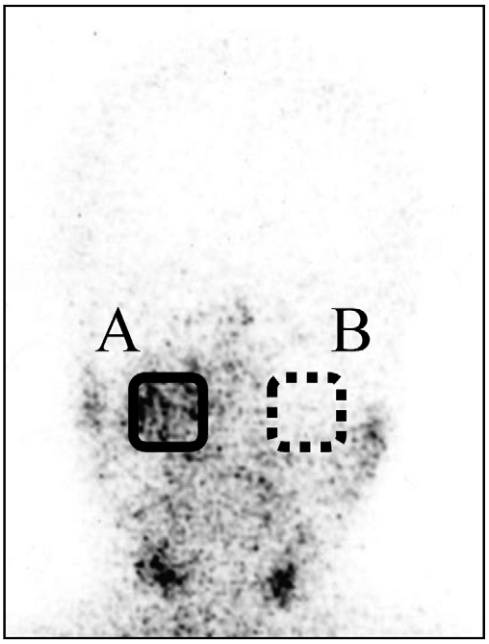


**Figure 1** Two regions of interest (ROI) on a frame image covered the tumor area (A) and the symmetrical region (B: control region).

injection) were carried out using a gamma camera. From the dynamic scan, 2-s scans were obtained continuously. A single 2-s scan constituted a frame data. Two regions of interest (ROI) on each frame covering both tumor and control areas were used to estimate the uptake of 201-Tl (Fig. 1). Early and delayed retention indexes were calculated from the results of each dynamic scan. The early retention index was the ratio of count of tumor to count of control in the early dynamic scan. The delayed retention index was the ratio of count of tumor to count of control in the delayed dynamic scan. From these two indexes, the tumor retention index was calculated; the ratio of the delayed retention index to the early retention index (Fig. 2). We used this tumor retention index for the evaluation of scintigraphy. The tumor retention index was compared with the histopathologic type and tissue differentiation.

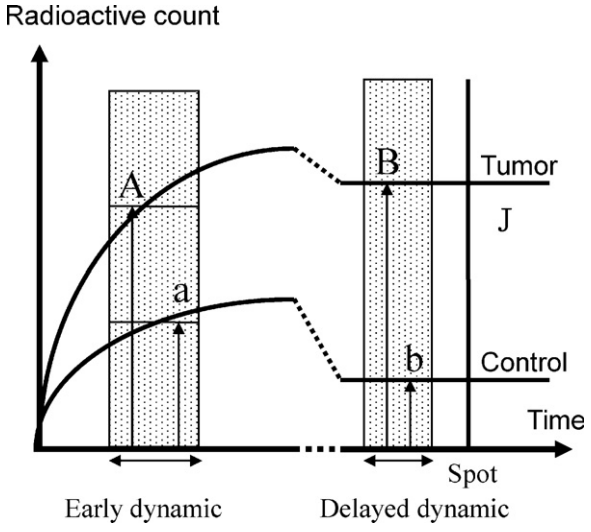


**Figure 2** Two curves showed radioactive count after injection in the tumor and control areas. In the dynamic scintigraphy, the early retention index was A/a and the delayed retention index was B/b. The tumor retention index was (delayed retention index)/(early retention index).

*(Results: tumor retention index of 201-Tl, histopathologic type and tissue differentiation)*. Tumor retention indexes varied widely ranging from 0.76 to 1.46 in patients. In the histopathologic type, the tumor retention indexes ranged from 0.76 to 0.93 (average was 0.82) in the benign group, and 0.78—1.46 (1.04) in the malignant group, respectively. In the tissue differentiation, tumor retention indexes ranged from 0.78 to 1.24 (average was 1.03) in the well group, from 0.91 to 1.42 (1.09) in the moderate group, and from 1.05 to 1.46 (1.24) in the poor group, respectively. We classified these tumor retention indexes into three groups: decreased ($<$0.9), unchanged (0.9—1.1), and increased ($>$1.1). The increased tumor retention index means that the washout of 201-Tl from tumor is delayed or the washout function is lost. On the other hand, the decreased tumor retention index indicates that the washout of 201-Tl is fast. As for histopathologic type, 80% of patients in the benign group belonged to the decreased tumor retention index group and no patient showed the increased. On the other hand, 28% and 67% of patients in the malignant group were included in the increased and unchanged groups. Only 5% of patients indicated the decreased. As for the tissue differentiation, 86% of patients in the poor group were included in the increased group and no patient showed the decreased. On the other hand, only 13% of patients in the well group belonged to the increased group (Table 1). These results showed that 201-Tl once taken up in malignant tumors had a tendency to remain.

**Accumulation of 201-Tl and $Na^+/K^+$-ATPase expression**

It was reported that the expression of $Na^+/K^+$-ATPase on cell membrane was one of the most important factors concerning the accumulation mechanism of 201-Tl in malignant tumors [14]. However, the role of $Na^+/K^+$-ATPase on the uptake mechanism of 201-Tl is not clearly understood, and there are few reports on tumors of the head and neck. In this section, we evaluated the role of $Na^+/K^+$-ATPase expression on 201-Tl scintigraphy of malignant tumors of the head and neck.

*(Methods and materials of immunohistochemistry for $Na^+/K^+$-ATPase expression)*. Sixty-five patients with malignant tumor of the head and neck (squamous cell carcinoma) and 22 patients with benign tumor were used.

Immunohistochemical staining was performed with tumor samples. Briefly, sections of tumors were treated with sodium citrate buffer, heated for the antigen retrieval, and then treated hydrogen peroxide for 10 min to block endogenous peroxidase activity. Sections were incubated with the primary and secondary antibody. After incubation, the sections were washed with Tris buffer saline, reacted with avidin-biotinylated-peroxidase complex, and stained with diaminobendizine [15]. $Na^+/K^+$-ATPase expression was graded into score 0 (stained under 5%), score 1 (from 5 to 49%), or score 2 (over 50%) [16] with reference to the negative and positive

**Table 1** Tumor retention index of 201-Tl, histopathologic type and tissue differentiation.

| Tumor retention index | Histopathologic type | | Tissue differentiation | | |
|---|---|---|---|---|---|
| | Benign | Malignant | Well | Moderate | Poor |
| | 10 patients | 85 | 53 patients | 25 | 7 |
| Decreased (<0.9) | 80% | 5 | 8% | 0 | 0 |
| Unchanged (0.9—1.1) | 20 | 67 | 79 | 56 | 14 |
| Increased (>1.1) | 0 | 28 | 13 | 44 | 86 |

**Table 2** Na+/K+-ATPase expression, histopathologic finding and tumor retention index.

| $Na^+/K^+$-ATPase | Histopathologic finding | | | | Scintigraphy | | |
|---|---|---|---|---|---|---|---|
| | Benign | Malignant | | | Tumor retention index | | |
| | | Well | Moderate | Poor | Decreased | Unchanged | Increased |
| | 22 patients | 32 | 27 | 6 | 24 | 24 | 17 |
| Score 0 | 32% | 44% | 0 | 16.7 | 41% | 4 | 12 |
| Score 1 | 59 | 40.5 | 56 | 16.7 | 45 | 58 | 35 |
| Score 2 | 9 | 15.5 | 44 | 66.6 | 14 | 38 | 53 |

controls. We compared $Na^+/K^+$-ATPase expression with the tumor retention index, histopathologic findings in malignant tumors of the head and neck.

*(Results: $Na^+/K^+$-ATPase expression, histopathologic finding and tumor retention index)*. As for the $Na^+/K^+$-ATPase expression and histopathologic finding, patients of the benign group showed score 0 (32%), score 1 (59%) and score 2 (9%). In the malignant group, scores 0, 1 and 2 were shown in 44%, 40.5% and 15.5% in the well group, respectively. Patients of the moderate group showed score 1 (56%) and score 2 (44%). In patients of the poor group, scores 0, 1 and 2 were shown in 16.7%, 16.7% and 66.6%, respectively. As for the $Na^+/K^+$-ATPase expression and tumor retention index, patients of the decreased group showed scores 0, 1, and 2 in 41%, 45% and 14%, respectively. Patients of the unchanged group revealed score 0 (4%), score 1 (58%) and score 2 (38%), respectively. In patients of the increased group, scores 0, 1 and 2 were observed in 12%, 35% and 53%, respectively (Table 2). The frequency of score 2 was elevated according as the tumor retention index became large. These results indicated that $Na^+/K^+$-ATPase expression was typical in malignant tumors and played the role of uptake of 201-Tl.

**Clinical evaluation of scintigraphy with 99m-Tc-MIBI**

This scintigraphic agent has been widely used to evaluate the viability of the myocardium, and the accumulation of this agent in malignant tumors has been also reported [17]. In this section, we evaluated the usefulness of 99m-Tc-MIBI scintigraphy for the diagnosis of malignant tumor of the head and neck.

*(Methods and materials of scintigraphy with 99m-Tc-MIBI)*. Nineteen patients with squamous cell carcinoma of the head and neck were used. The method of scintigraphy was almost the same as that of 201-Tl. Scintigraphy was performed with an intravenous injection of 600MBq of 99m-Tc-MIBI [18]. The tumor retention index was compared with the tissue differentiation.

*(Results: tumor retention index of 99m-Tc-MIBI and tissue differentiation)*. Retention indexes ranged from 1.1 to 3.1 in the early dynamic scan, and averages were 1.03 (well group), 1.8 (moderate) and 1.65 (poor). In the delayed dynamic scan, retention indexes ranged from 1.0 to 2.9, and averages were 1.1 (well group), 1.48 (moderate) and 1.27 (poor). From these retention indexes, tumor retention indexes were calculated. Tumor retention indexes ranged from 0.70 to 1.0, and averages were 0.91 (well group), 0.93 (moderate) and 0.79 (poor), respectively. Then, we classified grades of tumor retention indexes into >0.9 (slightly decreased), 0.9—0.8 (intermediately decreased) and >0.8 (severely decreased). Most of tumor retention indexes were under 1.0. We could find a decreasing tendency of tumor retention indexes from the early dynamic scan to delayed dynamic scan in malignant tumors of the head and neck. All patients in the well group

**Table 3** Tumor retention index of 99m-Tc-MIBI and tissue differentiation.

| Tumor retention index | Tissue differentiation | | |
|---|---|---|---|
| | Well | Moderate | Poor |
| | 7 patients | 8 | 4 |
| Slightly decreased (>0.9) | 71% | 12.5 | 0 |
| Intermediately decreased (0.9—0.8) | 29 | 50 | 50 |
| Severely decreased (<0.8) | 0 | 37.5 | 50 |
| % decrease from early to delayed (average) | 9% | 17.8 | 21 |

**Table 4** P-gp expression, tissue differentiation and tumor retention index.

| P-gp expression | Tissue differentiation (71 patients) | | | Tumor retention index (19) | | |
|---|---|---|---|---|---|---|
| | Well | Moderate | Poor | >0.9 (Slightly) | 0.9—0.8 (Intermediately) | <0.8 (Severely) |
| | 39 patients | 19 | 13 | 6 patients | 8 | 5 |
| Score 0 | 43% | 11 | 0 | 67% | 12.5 | 0 |
| Score 1 | 49 | 63 | 69 | 33 | 50 | 60 |
| Score 2 | 8 | 26 | 31 | 0 | 37.5 | 40 |

belonged to the slightly or intermediately decreased indexes. On the other hand, 50% of patients in the poor group showed the severely decreased index. The ''% decreases from the early to delayed tumor retention index'' were ranged from 0% to 30%, and the average of poor group was 21% (Table 3). These results revealed that 99m-Tc-MIBI once taken up in malignant tumors was discharged from tumors gradually, and this was opposite to 201-Tl.

**Accumulation of 99m-Tc-MIBI and P-gp expression**

99m-Tc-MIBI once accumulated is discharged gradually from tumors. This washout of 99m-Tc-MIBI from tumors is recognized with a tumor retention index, which is considered to depend on the expression of P-gp in tumor cell membrane [17,19]. Pg-p is observed on the cell membrane of both normal and tumor cells, and the expression is more distinct in malignant tumor cells [7]. However, there are few reports concerning the role of P-gp on Tc-99m-MIBI scintigraphy in malignant tumor of the head and neck. In this section, we evaluated immunohistochemically the level and role of P-gp in malignant tumors.

*(Methods and materials of immunohistochemistry for P-gp expression).* One group of 19 patients underwent both 99m-Tc-MIBI scintigraphy and an immunohistochemical examination. Moreover, another group of 71 patients underwent an immunohistochemical examination of P-gp expression.

Samples of malignant tumor were treated in citrate buffer to retrieve the antibody activity. They were incubated with $H_2O_2$, horse serum and a primary monoclonal antibody of JSB-1. They were incubated with secondary antibody solution, diaminobenzidine, $H_2O_2$, and peroxidase substrate solution. Finally, the nuclei were counter-stained with hematoxylin. In addition to these samples, we used three other tissue sections for the control of negative, a moderately positive and a severely positive stains [19]. We classified grades of staining of P-gp expression into score 0 (less than 5% of tumor cells), score 1 (5—50%) and score 2 (over 50%) [20]. We compared P-gp expression with the tissue differentiation and the tumor retention index in malignant tumors of the head and neck.

*(Results: P-gp expression, tissue differentiation and tumor retention index).* With respect to the P-gp expression and tissue differentiation in 71 patients, 43% and 49% of patients in the well group showed score 0 and 1. On the other hand, most patients in the poor group showed score 1 and score 2. No patient showed score 0. As for the P-gp expression and tumor retention index in 19 patients, 67% of patients in the slightly decreased group showed score 0, and no patient showed score 2. On the other had, 40% of patients in the severely decreased group showed score 2, and no patient showed score 0 (Table 4). These results indicated that P-gp expression was distinct in patients of low differentiation group and showed a well correlation with the discharge of 99m-Tc-MIBI.

**Comparison of 201-Tl with 99m-Tc-MIBI**

99m-Tc-MIBI and 201-Tl had each different uptake mechanism. 99m-Tc-MIBI accumulated distinctly in malignant tumors in the early phase, but the accumulation became less intense in the late phase. 201-Tl also accumulated in malignant tumors in the early phase, but the accumulation in the delayed phase of malignant tumors did not show any distinct decrease. In this section, we compared and evaluated the usefulness of 201-Tl and 99m-Tc-MIBI for the diagnosis of malignant tumors of the head and neck.

*(Results: diagnostic reliability of 201-Tl with 99m-Tc-MIBI).* The true positive, false positive, false negative, true negative, sensitivity, specificity and accuracy of the two scintigraphic agents are shown (Table 5). The sensitivity, specificity and accuracy of 201-Tl scintigraphy were 82.9%, 80.1% and 82.7%, respectively. On the other hand, the sensitivity and accuracy were 68.4% and 68.4% in 99m-Tc-MIBI scintigraphy. Thus, 201-Tl is a little superior to 99m-Tc-MIBI as an agent for malignant tumors of the head and neck.

## Scintigraphy for lymph nodes (lymphoscintigraphy)

99m-Tc-Re had been used once as agents for lymphoscintigraphy to detect metastatic lymph nodes. 99m-Tc-Re was composed of colloid of small particles (2—15 nm) [21,22] and a good radioactive agent for lymphoscintigraphy. Unfortu-

**Table 5** Diagnostic reliability of 201-Tl and 99m-Tc-MIBI.

| | 201-Tl | 99m-Tc-MIBI |
|---|---|---|
| True positive | 77% | 68 |
| False positive | 1 | 0 |
| False negative | 16 | 32 |
| True negative | 5 | 0 |
| Sensitivity | 82.9% | 68.4 |
| Specificity | 80.1 | — |
| Accuracy | 82.7 | 68.4 |

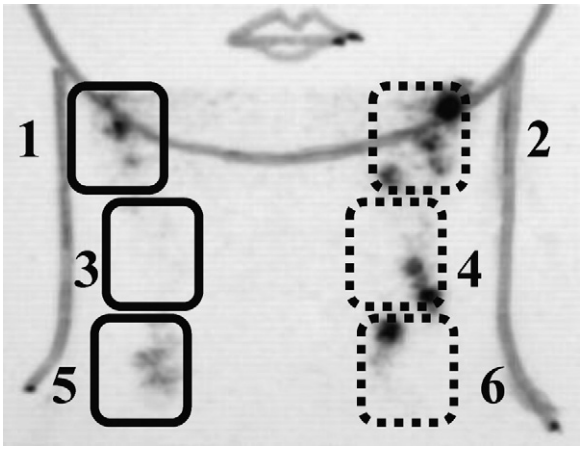


**Figure 3** Regions of interest (ROI) of cervical lymph nodes of dynamic lymphoscintigraphy. They were the right and left superior levels of internal jugular nodes (ROI numbers 1 and 2), the mid internal jugular nodes (3 and 4) and the inferior internal jugular nodes (5 and 6).

nately we cannot use this now in Japan because of legal problems. We had an opportunity before to use this as a part of clinical research of this agent. Injected 99m-Tc-Re was taken into small lymphatic vessels and this uptake chiefly depended on the pore size of the small lymphatic vessels. On the other hand, 99m-Tc-HSA-D consisted of dextran [23]. Generally, dextran at molecular weight of over 50,000 was taken into small lymphatic vessels [24]. The molecular weight of 99m-Tc-HSA-D was over 50,000, so this agent easily moved into small lymphatic vessels. Between 99m-Tc-Re and 99m-Tc-HSA-D, there was a difference in their mechanisms of uptake. Criterion for a metastasis of lymph nodes was based on the fact that normal lymph nodes could take 99m-Tc-Re or 99m-Tc-HSA-D, but metastatic lymph nodes decreased the uptake of them or could not take them. We performed two kinds of lymphoscintigraphy with them: a dynamic and a static lymphoscintigraphy.

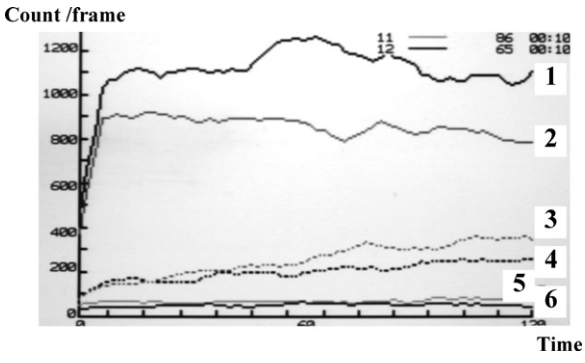


**Figure 4** Function curves of dynamic lymphoscintigraphy. These curves indicated the change in flow of the radioactive agent along the internal jugular chains. Curve numbers corresponded to ROI numbers of cervical lymph nodes respectively. Function curves showed various findings about changes in lymphatic system.

**Lymphoscintigraphy with 99m-Tc-Re**

We performed lymphoscintigraphy for finding out and diagnosing of metastatic lymph nodes from malignant tumors of the head and neck. Lymphoscintigraphy showed various images. Metastasis of lymph node usually caused a change of the flow rate in lymphatic vessels and a change of uptake of radioactive agent in lymph nodes. Therefore, lymphoscintigraphic images could show the change of lymph node function on the basis of the pathological change, and the change might be useful as a criterion for evaluation of lymph node metastasis. 99m-Tc-Re had been used in lymphoscintigraphy of internal jugular chains for a long time, but there were few clinical reports on this agent. In this section, we examined and evaluated the changes of internal jugular nodes due to metastases from malignant tumors of the head and neck by lymphoscintigraphy with 99m-Tc-Re.

*(Methods and materials of lymphoscintigraphy with 99m-Tc-Re).* Dynamic lymphoscintigraphy with Tc-99m-Re was evaluated on 17 patients with squamous cell carcinomas. Static lymphoscintigraphy was evaluated on 32 patients.

Dynamic lymphoscintigraphy was carried out immediately after the subcutaneous injection of 99m-Tc-Re (37MBq each) in both areas behind the ears simultaneously [9—11]. Twenty-second-scans were obtained continuously for 20 min. A 20 s-scan was recorded as a frame datum, and 60 frames were obtained. On each frame, six regions of interest covering both sides of internal jugular chains were used for evaluation of lymph node function (Fig. 3). Obtained frame data were used to make "Function curves" (Fig. 4). Function curves showed a lot of information about the lymphatic system. Furthermore, static images of lymphoscintigraphy were obtained 3 h after the injection.

*(Result-1: pathologic examination and dynamic lymphoscintigraphy with 99m-Tc-Re).* Ten of 17 patients were proved to be metastasis pathologically and they all showed a positive lymphoscintigraphic image (true positive). 7 of 17 patients were proved to be normal pathologically. 4 patients showed a positive lymphoscintigraphic image (false positive) and 3 patients revealed a negative image (true negative). The true-positive, false-positive, false-negative and true-negative were found in 71%, 29%, 0% and 100%. Then, the sensi-

**Table 6** Pathologic examination and dynamic lymphoscintigraphy with 99m-Tc-Re.

| Pathologic examination | | Dynamic lymphoscihoscintigraphy | |
|---|---|---|---|
| Metastasis | 10 patients | Positive | 10 patients |
| | | Negative | 0 |
| Normal | 7 patients | Positive | 4 patients |
| | | Negative | 3 |
| True positive | | 71% | |
| False positive | | 29 | |
| False negative | | 0 | |
| True negative | | 100 | |
| Sensitivity | | 100% | |
| Specificity | | 43 | |
| Accuracy | | 76 | |

**Table 7** Pathologic examination and static lymphoscintigraphy with 99m-Tc-Re.

| Pathologic examination | | Static lymphoscintigraphy | |
|---|---|---|---|
| Metastasis | 24 patients | Positive | 24 patients |
| | | Negative | 0 |
| Normal | 8 patients | Positive | 5 patients |
| | | Negative | 3 |
| True positive | | 83% | |
| False positive | | 17 | |
| False negative | | 0 | |
| True negative | | 100 | |
| Sensitivity | | 100% | |
| Specificity | | 38 | |
| Accuracy | | 84 | |

**Table 8** Pathologic examination and dynamic lymphoscintigraphy with 99m-Tc-HSA-D.

| Pathologic examination | | Dynamic lymphoscintigraphy | |
|---|---|---|---|
| Metastasis | 9 patients | Positive | 9 patients |
| | | Negative | 0 |
| Normal | 5 patients | Positive | 5 patients |
| | | Negative | 0 |
| True positive | | 64% | |
| False positive | | 36 | |
| False negative | | — | |
| True negative | | — | |
| Sensitivity | | 100% | |
| Specificity | | 0 | |
| Accuracy | | 64 | |

tivity, specificity and accuracy were 100%, 43% and 76% (Table 6).

*(Result-2: pathologic examination and static lymphoscintigraphy with 99m-Tc-Re)*. Twenty-four of 32 patients were proved to be metastasis pathologically and they all showed a positive lymphoscintigraphic image (true positive). 8 of 32 patients were proved to be normal pathologically. 5 patients showed a positive lymphoscintigraphic image (false positive) and 3 patients revealed a negative image (true negative). The true-positive, false-positive, false-negative and true-negative were found in 83%, 17%, 0% and 100%. Then, the sensitivity, specificity and accuracy were 100%, 38% and 84% (Table 7).

**Table 9** Pathologic examination and static lymphoscintigraphy with 99m-Tc-HSA-D.

| Pathologic examination | | Static lymphoscintigraphy | |
|---|---|---|---|
| Metastasis | 9 patients | Positive | 9 patients |
| | | Negative | 0 |
| Normal | 5 patients | Positive | 4 patients |
| | | Negative | 1 |
| True positive | | 69% | |
| False positive | | 31 | |
| False negative | | 0 | |
| True negative | | 100 | |
| Sensitivity | | 100% | |
| Specificity | | 20 | |
| Accuracy | | 71 | |

**Table 10** Diagnostic reliability of 99m-Tc-Re and 99m-Tc-HSA-D.

| Lymphoscintigraphy | | Statistical data | | |
|---|---|---|---|---|
| | | Sensitivity | Specificity | Accuracy |
| 99m-Tc-Re | Dynamic | 100% | 43 | 76 |
| | Static | 100 | 38 | 84 |
| 99m-Tc-HSA-D | Dynamic | 100% | 0 | 64 |
| | Static | 100 | 20 | 71 |

**Lymphoscintigraphy with 99m-Tc-HSA-D**

Lymphoscintigraphy with 99m-Tc-HSA-D might be somewhat different from 99m-Tc-Re in findings depending on the different component from that of 99m-Tc-Re [24].

*(Methods and materials of lymphoscintigraphy with 99m-Tc-HAS-D)*. Dynamic and static lymphoscintigraphy with 99m-Tc-HSA-D were performed in 14 patients with squamous cell carcinoma of the head and neck.

We injected 74MBq of 99m-Tc-HSA-D subcutaneously in both areas behind ears. Dynamic and static lymphoscintigraphy were carried out. The criteria of metastasis were almost the same as those of 99m-Tc-Re.

*(Result-1: pathologic examination and dynamic lymphoscintigraphy with 99m-Tc-HSA-D)*. Nine of 14 patients were proved to be metastasis pathologically and they all showed a positive lymphoscintigraphic image (true positive). 5 of 14 patients were proved to be normal pathologically. They all showed a positive lymphoscintigraphic image (false positive). The true-positive and false-positive were found in 64% and 36%. Then, the sensitivity and accuracy were 100% and 64% (Table 8).

*(Result-2: pathologic examination and static lymphoscintigraphy with 99m-Tc-HSA-D)*. All patients proved to be metastasis pathologically showed a positive lymphoscintigraphic image (true positive). 4 of 5 patients proved to be normal pathologically showed a positive lymphoscintigraphic image (false positive) and 1 patient revealed a negative image (true negative). The true-positive, false-positive, false-negative and true-negative were found in 69%, 31%, 0% and 100%. Then, the sensitivity, specificity and accuracy were 100%, 20% and 71% (Table 9).

**Comparison of 99m-Tc-Re with 99m-Tc-HSA-D**

99m-Tc-Re was composed of uniform particles of a suitable size for the pores. 99m-Tc-Re flew through small lymphatic vessels, then reached lymph nodes. On the other hand, 99m-Tc-HSA-D did not consist of any colloidal particles [10,11],

therefore the mechanism of uptake of this radioactive agent in lymph nodes was very different from that of 99m-Tc-Re, and because of this their lymphoscintigraphic patterns were assumed to be different in some degree.

*(Results: diagnostic reliability of 99m-Tc-Re and 99m-Tc-HSA-D)*. The sensitivity, specificity and accuracy of lymphoscintigraphy with 99m-Tc-Re and 99m-Tc-HSA-D were shown in Table 10. In comparison of 99m-Tc-Re with 99m-Tc-HSA-D, 99m-Tc-Re showed a high accuracy both in the dynamic and static lymphoscintigraphy, and was estimated to be superior to 99m-Tc-HSA-D as an agent for lymphoscintigraphy. This might depended on the component of each agent: 99m-Tc-Re was consisted of colloid and 99m-Tc-HSA-D was dextran.

## Discussion

We made re-evaluation of some of our previous reports [3—11] on scintigraphy for malignant tumors and lymph node metastasis. There were some clues to find a solution to problems in scintigraphy. The results in this article indicated a possible hint to make a qualitative diagnosis of malignant tumors or to differentiate malignant tumors from inflammatory lesions. For example, tumor retention indexes showed different tendencies between malignant lesions and benign lesions including inflammatory changes, or the level of transport proteins on cell membrane have a possible clue to reveal grades of tissue differentiation of tumors like tumor markers.

### Scintigraphy for tumors with 201-Tl and 99m-Tc-MIBI

Both 201-Tl and 99m-Tc-MIBI are now rarely used for diagnosis of malignant tumors of the head and neck [8] because FDG-PET has been widely introduced for the same purpose [1]. However, 201-Tl and 99m-Tc-MIBI have some advantages to FDG-PET, for example, transport proteins ($Na^+/K^+$-ATPase for 201-Tl and P-gp for 99m-Tc-MIBI) were helpful for qualitative diagnosis and have a possibility to become factors like tumor markers. In addition, 201-Tl and 99m-Tc-MIBI are not so expensive. In this article, we re-evaluated retrospectively the usefulness of 201-Tl and 99m-Tc-MIBI for a diagnosis of tumors of the head and neck. We could obtain important information from dynamic scintigraphy. In the early phase, both 201-Tl and 99m-Tc-MIBI accumulated well in viable tumor cells [12,18], although they have physical differences. $Tl^+$ has physical effects similar to $K^+$ and is taken up actively because it has an ion radius similar to $K^+$, and malignant tumors need a large amount of $K^+$ [25,26]. On the other hand, 99m-Tc-MIBI accumulated in tumor cells by plasma membrane potentials [6]. With respect to the accumulation mechanism in the delayed phase, we performed some evaluations and obtained some useful results. 99m-Tc-MIBI first reached tumor cells through the tumor vascular system and was taken into tumor cells by plasma membrane potentials. Next, the accumulated 99m-Tc-MIBI was discharged from tumor cells by P-gp expressed on the cell membrane which was well known as a responsible protein in the multi-drug resistance [27]. On the other hand, 201-Tl was first brought to tumor cells like 99m-Tc-MIBI, and the accumulation in tumor cells was increased by the active transportation with $Na^+/K^+$-ATPase expressed on the cell membrane [28]. In our investigation, the accumulation of 201-Tl in the delayed phase correlated well with $Na^+/K^+$-ATPase [5]. As for the relationship with the tumor retention index, the tissue differentiation and tumor retention index showed an evident correlation. This suggested that tumor retention indexes correlated with transport proteins. Tomura et al. [29] reported a tendency that the tumor retention index of malignant tumors decreased in 99m-Tc-MIBI scintigraphy. They reported an about 30% decrease. On the other hand, Tonami et al. [30] reported a decreased tumor retention index of 4.6—6% in benign tumors, and demonstrated an increase of more than 20% in malignant tumors in 201-Tl scintigraphy. Thus, the tumor retention index decreased with 99m-Tc-MIBI and increased with 201-Tl when tumors were malignant [31]. In this article, we showed the usefulness of 99m-Tc-MIBI and 201-Tl, especially we showed that the tumor retention index showed a good correlation with the grade of tumor malignancy, and that the accumulation chiefly depended on transport proteins of $Na^+/K^+$-ATPase and P-gp [5,32,33].

### Scintigraphy for lymph nodes with 99m-Tc-Re and 99m-Tc-HSA-D

Lymphoscintigraphy is now performed for detecting sentinel lymph nodes of malignant tumors [34]. However, we had performed this method for detecting and diagnosing metastatic lymph nodes from malignant tumors of the head and neck [9—11]. For the purpose of those, we used two kinds of agents: 99m-Tc-Re and 99m-Tc-HSA-D. Between 99m-Tc-Re and 99m-Tc-HSA-D, there is a difference in the mechanism of uptake because these two agents are composed of different components [9,23,24]. In this article, we re-evaluated retrospectively the usefulness of lymphoscintigraphy with 99m-Tc-Re and 99m-Tc-HSA-D and compared the results each other. We carried out two types of scintigraphies: a dynamic and astatic lymphoscintigraphy. In dynamic lymphoscintigraphy, both 99m-Tc-Re and 99m-Tc-HSA-D showed no false negative, but showed false positive. This result might depend on the fact that lymphatic drainages easily changed even if the pathology of lymph nodes was benign or malignant, and usually showed variable pattern. Sometime lymphatic drainages change even in normal conditions. The accuracy of diagnosis was 76% in 99m-Tc-Re and 64% in 99m-Tc-HSA-D respectively, but their specificities were not high. In static lymphoscintigraphy, the accuracy was 84% in 99m-Tc-Re and 71% 99m-Tc-HSA-D, but their specificities were as low as those dynamic scintigraphy. The low specificities might depend on the fact that lymph nodes might show changes in advance to metastasis, for example inflammatory effects from tumor tissues. Moreover, it might be a cause to the low specificity that it was impossible to let all lymph nodes have one to one correspondence to each other between lymphoscintigraphy and pathologic examination. In comparison of 99m-Tc-Re with 99m-Tc-HSA-D, 99m-Tc-Re showed a slightly higher agreement with pathological findings than Tc-99m-HSA-D. Unfortunately, it is difficult now to find out a single lymph node metastasis or a micro metastasis. These results of our evaluation of dynamic and static lymphoscintigraphy might be a hint to solve problems.

## Summary

At the present time that 201-Tl, 99m-Tc-MIBI, 99m-Tc-Re and 99m-Tc-HSA-D become not to be used popularly in comparison with FDG-PET, we do not expect that our previous results are useful or helpful to the routine dental practice directly. However, FDG-PET is recently found to have a problem in diagnosis of malignant tumors, for example FDG-PET accumulates both in malignant tumors and inflammatory lesions. This is just the problem that we also tried to resolve until now. Therefore, we hope that even a small part of our results shown in this article could be a clue or hint for dentists to try to find out a solution of problem, if it is a very small help.

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